IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Rajendra S. Bhatnagar Serial No.: 08/278,878 Filed: July 22, 1994 For: SYNTHETIC COMPOUNDS AND COMPOSITIONS WITH ENHANCED CELL BINDING	Group Art Unit: 1811 Examiner: Avis M. Davenport San Francisco, California

Hon. Commissioner of
Patents and Trademarks
Washington, D.C. 20231

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on February 5, 1996.

Sarah Lyn Billingsley 2/5/96
Signature Date

DECLARATION OF RAJENDRA S. BHATNAGAR

I, Rajendra S. Bhatnagar, hereby declare and state as follows:

1. I am the inventor of the subject invention, and am a Professor of Biochemistry and Oral Biology in the Laboratory of Connective Tissue Biochemistry at the University of California School of Dentistry at San Francisco, California.

2. I hold a Ph.D. in Biochemistry awarded by Duke University.

3. The following experiments described as "Example B" were performed under my direction and control.

EXAMPLE B

MINIATURE SWINE SUBCUTANEOUS MODEL

Method

The inventive peptide P15 was covalently linked to methylcellulose (in a manner analogous to Example 6 of the specification) and placed in a Hunt Schilling chamber which was lined with a PGA mesh. The chamber was constructed from a stainless steel wire mesh 2.5 cm long and 0.5 cm in diameter. The implants were placed subcutaneously in the backs of Yucatan miniature swine, and extracted at 6, 9, and 14 weeks. The control condition used the identical materials, but without the P15 present.

Results

Figure 1A shows the control condition at 6 weeks. This showed an inflammatory response, with many multi-nucleated giant cells.

Control Figure 1A of Example B

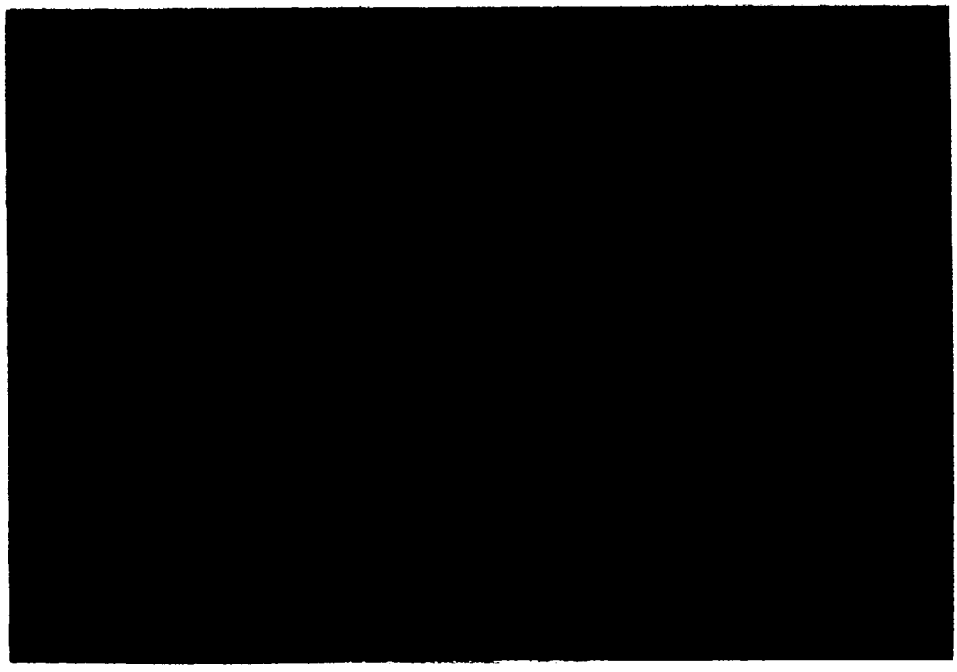


Figure 1B shows the inventive embodiment implant after 6 weeks. There was no sign of inflammation present. The sample showed a well organized connective tissue, with abundant healthy fibroblasts.

Inventive Embodiment of Figure 1B of Example B

I further declare that all statements made on my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent resulting therefrom.

Dated: January 19 1996.

RAJENDRA S. BHATNAGAR

under the blue light to obtain the extent of bone ingrowth into the defect at 14 days. Also following histologic staining, the extent of stained linear bone ingrowth was measured in a similar fashion.

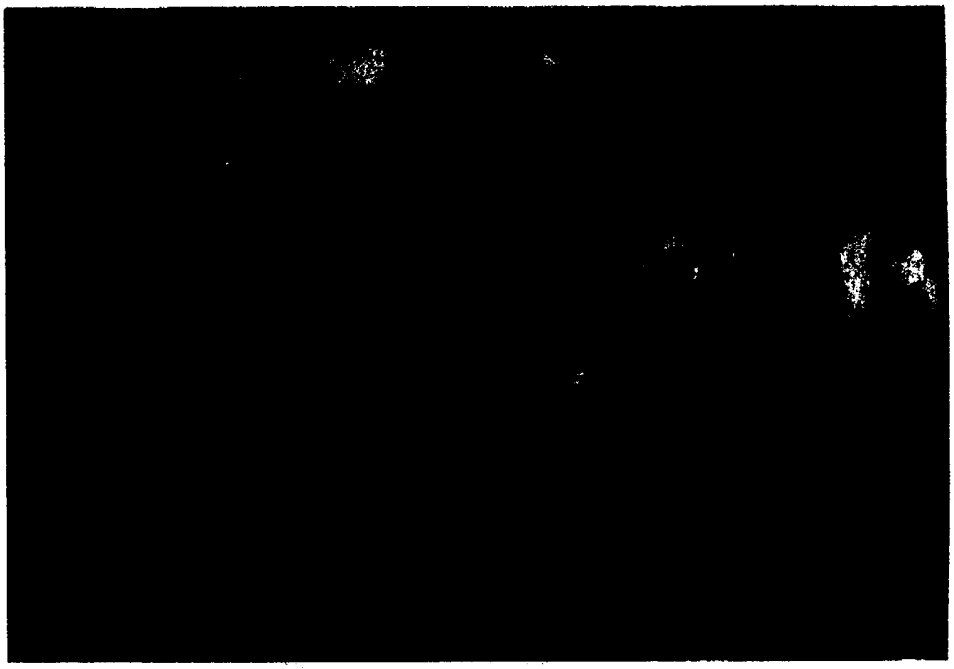
Results

All animals recovered from the surgery and went to term. In all cases, implant materials were well accepted with no evidence of prolonged inflammatory response or fibrous tissue encapsulation.

Using stained bone sections for bone measurement, the inventive implant demonstrated a statistically significant mean increase in bone of 40.2%.

The control particles showed minimal bone formation in the center of the defect (see Figure 1A).

(Control) Figure 1A of Example A



By contrast, the occurrence of bone in the center of the defect, as well as the bridging effect of particles that were not touching, indicates the osteo-inductive nature of the inventive peptide (see Figure 1B).

Inventive Embodiment Figure 1B of Example A

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Sarah Lyn Billingsley 2/5/96
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DECLARATION OF ANDREW J. TOFE

I, Andrew J. Tofe, hereby declare and state as follows:

1. I am the President and CEO of CeraMed Corporation, with a place of business at Lakewood, Colorado. CeraMed Corporation is a licensee of the subject patent application.

2. I hold a Ph.D. in Chemistry awarded by Florida State University.

3. I am a principal investigator engaged in *in vivo* safety studies of the invention and for the following described rabbit studies, set out as "Example A".

EXAMPLE A

OSSEOUS DEFECT ANIMAL STUDIES

Method

Ten NZW rabbits were utilized to test the healing potential of inventive implants in a bony defect site. The healing response to the materials was examined at two weeks. Bilateral 8-mm diameter circular defects were created in the parietal bone of the skull on either side of the sagittal suture using an 8-mm outer diameter trephine. Care was taken to leave the dura intact. One defect was filled with porous hydroxylapatite ("HA") granules prepared with an inventive peptide ("P15") by CeraMed, prepared as an inventive embodiment termed "ABM", or Anorganic Bone Mineral. ABM in a particulate form exists as smooth irregular shaped porous particles. For studying the effect of the inventive P-15 peptide on cell attachment, the peptide was adsorbed on ABM by incubating ABM for 24 h in a solution of the peptide in phosphate buffered saline (PBS) in a ratio of 1.0 g ABM: 2.0 ml solution. The incubation was carried out at room temperature with gentle shaking to ensure equilibration of the peptide with all available surface on the microporous ABM. Following incubation, ABM was washed three times with 5X volume of PBS to remove unadsorbed peptide. The ABM powder was collected and dried in a desiccator over Drierite.

The contralateral side of the defects were filled with HA alone. In an effort to measure the amount of bone formation at multiple time periods, each animal was fluorescently labelled with oxytetracycline, at 10 days, and 2-4-*bis*[N,N'-Di-(Carboxymethyl)-Aminomethyl] Fluorescein (DCAF) at 14 days.

At sacrifice the defects were examined grossly and explanted. Samples were fixed in 40% ethanol and then dehydrated in increasing concentrations of ethanol to 100%, followed by infiltration and embedding in polymethylmethacrylate. Once polymerized, sections were cut using a low speed diamond saw. In order to obtain the most information, the cranial samples were first cut along the diameter of the 8-mm trephine defect. Next, one-half of each sample was sectioned in the coronal plane, while the remaining half was sectioned in the horizontal plane. The coronal sections illustrated the distribution of bone from the inner table of the skull to the outer table, while the horizontal sections showed bone formation from the edge of the defect toward the center. For illustrative micrographs, samples were first stained with Stevenel's blue and then counterstained with Van Gieson picro-fuchsin.

Analysis included a linear measurement of the extent of ingrowth into the defect site of new trabecular bone at 10 days and 2 weeks, as well as a measurement of the area of new bone expressed as a percentage of the total defect area.

For evaluation of the linear extent of bone ingrowth into the defect, samples were viewed under ultraviolet light. The distance from the defect margin to the leading edge of the 10-day tetracycline label was measured from each side of the defect using a micrometer eyepiece. The two sides were added and the total ingrowth distance normalized by dividing by the total defect width measured at the time of evaluation. This yielded a linear measure of the extent of ingrowth expressed as a percent of the defect diameter. Similar measurements were made using the DCAF label

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Dated: 1/23/96.

ANDREW J. TOFE